An Investigation of a Sono-Chemical Approach In Sterilization Problems

Second Semi-Annual Progress Report
1 January 1965 to 1 July 1965

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Grant No. NsG-684

Submitted to:
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1. Purpose of Investigation.

As stated in the first semi-annual report (1 July 1964 - 1 January 1965), it is the object of the present investigation to determine the feasibility of techniques designed to reduce the time required for the sterilization of space vehicles. Specifically, it is planned to determine the effect of airborne sonic and ultrasonic irradiations on the viability of appropriate test microorganisms. In addition, it is proposed that the combined effect of gaseous sterilizing agents, such as ethylene oxide, and acoustical vibrations on the survival of bacterial spores be explored.

2. Effect of Sonic and Ultrasonic Irradiations on the Spores of Bacillus subtilis var niger.

Details of experimental materials and conditions are listed in the first semi-annual report (1 July 1964 - 1 January 1965). A schematic diagram of the test chamber employed in this investigation is illustrated in Figure 1.

To determine the effect of sonic and ultrasonic irradiations alone on the viability of B. subtilis var niger, spores of the latter microorganism, distributed on filter paper strips, were irradiated at two frequencies, 9.9 and 30.4 kc/sec respectively. Two different transducers were employed to obtain the desired frequencies. The spore-impregnated paper strips were placed at distances of 0.5, 2, 3.5, and 4 inches from the end of each transducer. All tests were run at 40 C and the humidity was adjusted to an initial value of 40%. Irradiation periods ran from 1 to 6 hours. At 9.9 kc/sec, [illegible] significant results, as determined by Student's "t" test (control

level = 99%), were obtained at the longer periods of irradiation. The results may be summarized as follows;

Hours	Distance From Transducer, inches			
	9.5	5.0	3.5	1.0
1	-	-	-	-
2	-	-	-	-
4	-	-	-	+
8	+	+	+	+

+ = statistically significant values

- = statistically non-significant values

The transducer employed above delivers 0.123 watt/cm^2 over an area of 7 cm^2.

At a frequency of 30.4 kc/sec, more effective kill, as evidenced by a larger number of significant values, was obtained. The temperature and humidity were set at 40 C and 40%, respectively. The results obtained were as follows;

Hours	Distance From Transducer, inches			
	9.5	5.0	3.5	1.0
1	-	-	-	-
2	-	-	-	+
4	-	+	+	+
8	+	+	+	+

+ = statistically significant values

- = statistically non-significant values

The transducer, in this case, yields 0.35 watt/cm^2 over an area of 2.5 cm^2.

The data collected demonstrates that airborne sonic and ultrasonic irradiations may be employed for killing microorganisms. It is apparent that the irradiation time, and the distance of the test sample from the transducer, are important factors in achieving statistically significant kill. Other factors, not yet fully investigated, are the effects of temperature and humidity on the antimicrobial activity of sound. At the present time, ultrasonic frequencies appear to be more effective than those in the sonic range. It appears unlikely that the destruction of bacterial spores by sound waves transmitted in air is caused by cavitation effects. Rather, tentatively attributing death to "agitation" brought about by sound appears more attractive. The results obtained in this investigation assume importance when it is recalled that air is an effective sound barrier. Rheins and Finlay (1954) reported that the acoustic reflection factor for air is 96%. It is obvious that the work being performed in the present investigation represents a pioneer effort in a field largely unexplored.

3. Effect of Ethylene Oxide and Ultrasonic Irradiation.

A series of experiments employing ethylene oxide, at various concentrations, in combination with sound were completed. Statistically significant results (99% certainty) were obtained at ethylene oxide concentrations of 250 and 150 mg/liter, respectively. In each case, the spores of B. subtilis var niger were also exposed to ultrasonic irradiations at a frequency of 30.4 kc/sec. The results with ethylene oxide, at a level of 250 mg/liter, and ultrasound were as follows:

Sound (kc/sec)	Ethylene Oxide (mg/liter)	Plate Count
None	250	810
None	250	380
None	250	345
None	250	71
30.4	250	3
30.4	250	1
30.4	250	2
30.4	250	2

Relative humidity = 40%; Temperature = 50° C.
Irradiation time = 40 minutes. Samples placed 1 inch from transducer.

More dramatic results were realized when the ethylene oxide level was reduced to 125 mg/liter. The enhancement of ethylene oxide activity by ultrasound was obvious from the data collected which is shown below:

Sound (kc/sec)	Ethylene Oxide (mg/liter)	Plate Count
None	125	6245
None	125	2230
None	125	205
None	125	3950
30.4	125	4
30.4	125	3
30.4	125	1
30.4	125	4

Relative humidity = 40%; Temperature = 50° C.
Irradiation time = 40 minutes. Samples placed 1 inch from transducer.

In both cases, the combined action of ethylene oxide and ultrasound was greater than the action of ethylene oxide alone.

4. Future Effort.

In order to minimize possible variations between duplicate experimental designs it is planned to make improvements on the test chamber being used. A direct read-out humidity gauge will be installed so that humidity can be monitored over long periods of time. In addition, another heater will be added to the system in order that experiments to be run at 60 C. can be easily carried out. A membrane filter designed to continually sterilize the air recirculated in the chamber will be installed.

Experimentally, the effect of sound alone on spores of B. subtilis var. niger will be investigated at higher temperatures. This effort will be expanded to include a study of the combined effects of ethylene oxide and sound at temperatures up to 60° C. Other variables, such as the role of humidity in the lethal action of ethylene oxide and sound will be investigated.

5. Literature Cited.

Rheins, M. and Finlay, J. 1954. The effect of ultrasonic irradiation on certain properties of influenza virus. J. Infectious Diseases, 95: 79-85.

6. Publication

It is expected that the data reported above, and related work, will be submitted for publication in a scientific journal within the next six months.

SCHEMATIC DIAGRAM OF
SONICATION CHAMBER

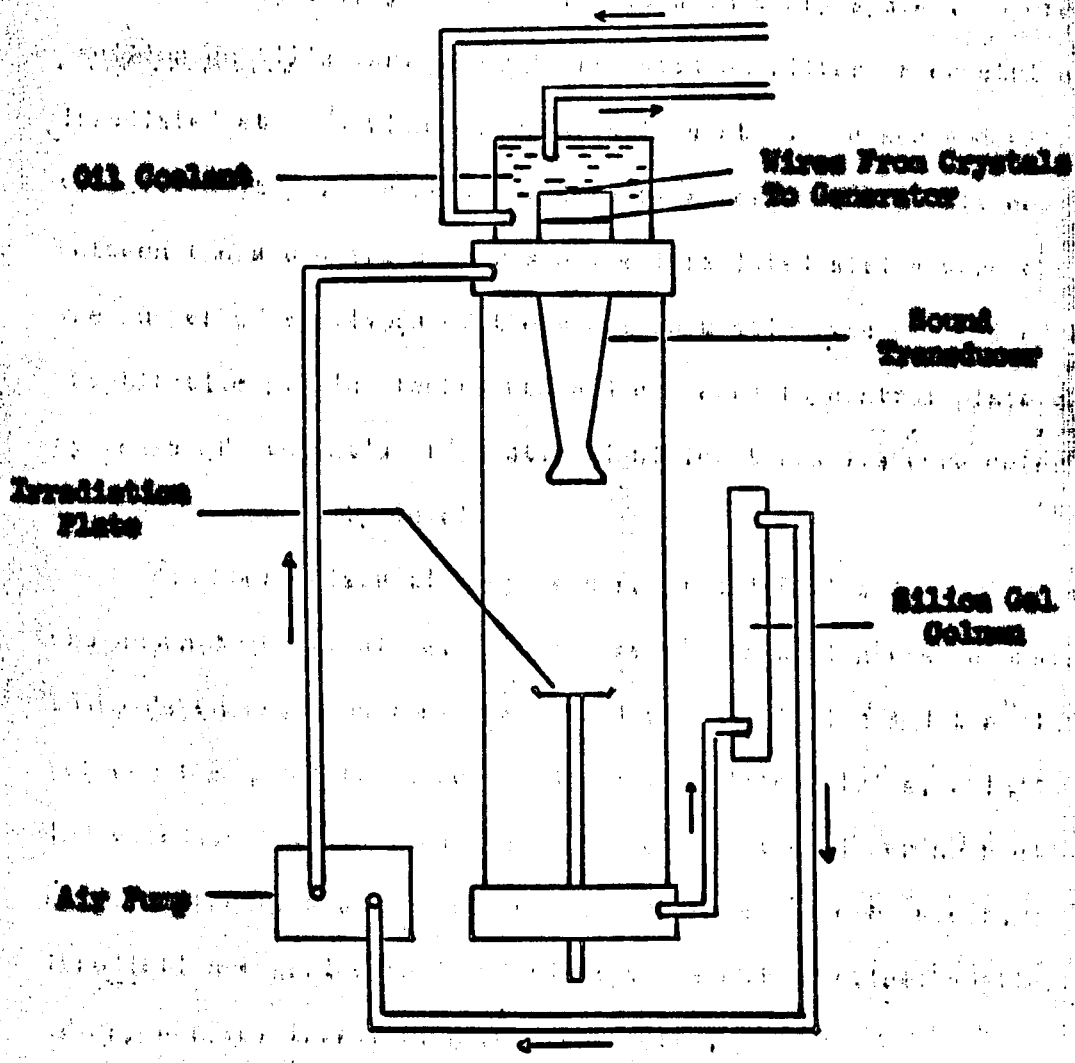

Figure 1